

THE RESPONSE OF PREPUBERAL HEIFERS TO NORGESTOMET
AND/OR FOLLICULAR FLUID AND THE INDUCTION OF
ESTRUS IN OVARIECTOMIZED COWS WITH
SYNCRO-MATE B

by

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A THESIS

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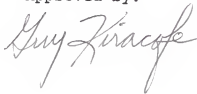
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A handwritten signature in cursive script, appearing to read "Guy Ziracofe".

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LITERATURE REVIEW

PUBERTY

Puberty in the bovine is influenced by many factors, including breed, body condition, plane of nutrition, photoperiod and temperature.

Age at puberty is different among breeds. The first ovulation occurs earlier in dairy heifers than in beef heifers, and heifers of Zebu origin reach puberty later in life than Bos tarus heifers (Joubert, 1963). In women, body condition (percentage of body fat) influences the onset of puberty, and Frisch (1984) suggested that a particular threshold level of fat to lean mass is normally needed for puberty. In heifers fed varying levels of energy (80 to 120% of NRC requirements), estimates of body composition were useful in predicting weight at puberty, but did not support the theory of a critical weight or body composition in heifers (Brooks et al., 1985). Heifers fed either a low or high plane of nutrition differed in average age at puberty by 271 d (Joubert, 1963). Protein content of the diet affected age at puberty in Zebu heifers fed isocaloric diets that varied only in protein content (Oeydiipe et al., 1982). Heifers on high, medium, or low protein diets

reached puberty at average ages of 570.4, 640.8, and 704.2 d, respectively. Photoperiod also may be involved in the attainment of puberty, because fall-born heifers reach puberty at

younger ages than spring-born heifers. Heifers raised under simulated spring-fall conditions (both spring and fall born) reached puberty earlier than heifers raised under simulated fall-spring conditions (Schillo et al., 1983). Presence of mature males does not appear to influence the onset of puberty in heifers as it may in some species (Roberson et al., 1987), but there have been conflicting results on the influence of mature cows on age at puberty, seemingly dependent on breed (Nelsen et al., 1985). Although many factors, such as those listed above, can influence the timing puberty, certain endocrine events must occur regardless of the age, season, weight, genetic makeup or body condition of the animal.

ENDOCRINE MECHANISMS INVOLVED IN THE ONSET OF PUBERTY

The relationship of pituitary and gonadal hormones at the time of puberty have been investigated thoroughly. Just before the onset of puberty, secretion of LH and FSH in heifers increases. Although the frequency of pulses of LH and FSH remain constant from 1 to 10 mo of age, the pituitary appears capable of responding to GnRH at all ages

(Schams et al., 1981). In experiments conducted by Gonzalez-Padilla et al. (1975) blood samples collected from 30 to 60 d before the first preovulatory peak of LH showed no marked changes in concentrations of FSH, PRL, or GnRH at the onset of puberty or during the first cycle. The significant changes observed during the 30 to 60 d before the first preovulatory LH surge were: (1) LH concentrations were higher than those observed after the preovulatory LH surge and a peak was observed in all heifers between 9 and 11 d before the preovulatory LH surge, and (2) progesterone levels were low, but a distinct elevation occurred preceding both LH peaks in all heifers. A different mode of LH secretion appears to occur as in transition from a prepuberal to postpuberal period. concentrations of estradiol in plasma remained relatively constant during this period. More heifers which express behavioral estrus at puberty exhibit a pre-estrual elevation in serum progesterone concentrations than heifers which fail to show estrus at puberty (Rutter and Randel, 1986). The source of this prepubertal progesterone in heifers was determined to be luteal tissue located beneath the surface of the ovary, not palpable or grossly observable on the surface of the ovary (Berardinelli et al., 1979). At least in the ewe lamb, this transient luteal structure is not essential for sexual maturation and its lifespan is uterine dependent (Keisler et al., 1983). These prepuberal rises in

progesterone and transient luteal structures are probably artifacts of the maturing hypothalamic-pituitary axis, and the onset of controlled pulsatile secretion of LH. Changes in the mode of LH secretion in the presence of relatively constant levels of estradiol-17 β represent the diminished negative feedback effects of estradiol-17 β on the secretion of LH (Pelletier et al., 1981; Ramaley, 1979). In ewe lambs which were ovariectomized prior to puberty (11 wks) and implanted with silastic implants containing crystalline estradiol, serum LH was suppressed to undetectable levels until the time at which first ovulation normally occurs in untreated controls (Foster and Ryan, 1979). Despite constant levels of estradiol, LH increased at this time to castrate levels. In the lamb, a stimulus to the tonic secretion of LH occurs at puberty that appears to be independent of steroidal control. Ovariectomized lambs not treated with estradiol implants maintained low castrate levels of serum LH until a similar time of puberty in intact lambs, then a secondary rise equal to adult castrate concentrations of LH occurred. An increasing threshold to the negative feedback effect of estradiol on LH secretion is an age related response (Schillo et al., 1982). In ovariectomized heifers treated with either .2 or 2.0 mg estradiol/kg body weight at 4, 8, or 12 mo of age, those treated at 4 mo of age exhibited a lower frequency of LH pulses than those treated at either 8 or 12 mo of age. In

addition, heifers treated at 4 mo exhibited a longer suppression of pulsatile LH release than the older heifers. Results also indicated a positive relationship between the dose of estradiol-17 β and the duration of LH suppression. These results indicate that estradiol-17 β inhibits LH secretion by effecting its pulsatile mode of secretion, and that this negative feedback effect of estradiol on LH secretion has a threshold level that increases as heifers approach puberty.

It appears that LH pulse frequency must increase, for puberty to occur and this may be modulated by a preovulatory progesterone stimulus. Follicular growth must occur and may require FSH secretion at levels higher than those before the time of puberty. The preovulatory follicle must reach a size where it is capable of producing estrogen.

INDUCTION OF PUBERTY

Attempts to induce puberty generally have involved giving injections or infusions (either continuous or pulses) of LH-RH, and injections or implants of estrogens and progestogens given separately or in combination. Skaggs et al. (1986) gave pulses or infusions of LH-RH to prepuberal heifers. Although they were not successful in inducing puberty they did induce preovulatory-like releases of LH and prepubertal rises in progesterone in some animals. Numerous

attempts have been made to control and induce ovulation in cows and heifers with progesterone. Progesterone containing intravaginal devices placed in prepuberal heifers for 3 d resulted in seven of nine 15-mo old heifers (334 kg) in estrus within 4 d after removal of these devices. Ovulation accompanied estrus in six of the seven heifers (Scheffield and Ellicott, 1982). Thirty-two prepuberal holstein heifers 6 to 8 mo of age (250 kg) received various types of sc implants or a vaginal coil containing progesterone for 20 d (Rajamahendran et al., 1982). Location of the progesterone-containing device had no effect on serum concentrations of progesterone, but the amount of surface area was related to its effectiveness. At implant removal, heifers recieved either saline, 5 mg estradiol-17 β , 500 IU PMSG, or 100 ug GnRH. Of the saline, estradiol-17 β , PMSG and GnRH treated heifers, 0, 5, 6, and 2 exhibited estrus and 0, 2, 6, and 2 ovulated, respectively. Of those heifers which exhibited estrus and ovulated, only one heifer in each of the treatment groups returned to estrus 20 to 23 d after the induced estrus. The authors suggested that while none of the heifers treated with saline after implant removal exhibited estrus or ovulated, the progesterone treatment must be followed by an ovulatory hormone stimulation to induce estrus and ovulation in prepuberal heifers, because this priming results in a greater response to treatment with

stimulation by an ovulatory hormone. Even then, only a few heifers continued to cycle.

The effect of injections of progesterone and estradiol-17 β on serum LH, FSH, and ovarian activity was examined in a trial where half sib prepuberal angus heifers were allotted randomly to receive either 2 mg estradiol-17 β injected im on d 0 or 20 mg progesterone im on d -2 or a combination of both treatments (Gonzalez-Padilla et al., 1975b).

Progesterone neither induced estrus nor had a significant effects on concentrations of LH or FSH in serum. Of the estradiol and progesterone-estradiol treated heifers, only one of six heifers in each group exhibited estrus. In these two groups, estradiol injection elicited an LH peak 12 to 18 h after injection, but no distinct FSH peak. In the progesterone-estradiol group, heifers developed CL, exhibited elevated serum progesterone between d 8 to 13 after estrus, and returned to estrus 19 to 21 d later. The two heifers treated with estradiol did not develop CL, and failed to return to estrus within 30 d after treatment. It was concluded that the increased response to endogenous LH by the ovaries of prepuberal heifers, occurred only in those that had been primed with progesterone.

Short et al. (1976) showed that more prepuberal heifers (8.5 mo old and 249 kg) given a silastic implant with progesterone for 6 d plus an injection of estradiol-17 β 24 h after implant removal showed estrus and ovulated within 4 d

than heifers treated with estradiol-17 β . The combination treatment also induced estrus in more heifers than progesterone alone. In another trial, the same researchers showed a 9-d treatment with progesterone in prepuberal heifers (13.7 mo old and 323 kg body wt) induced estrus in as many heifers as in those given 9 d of norgestomet plus 5 mg estradiol-17 β 24 h after implant removal. Eleven of 23 heifers given norgestomet plus estradiol had short or split cycles of less than 4 d.

Gonzalez-Padilla et al. (1975c) also utilized progesterone or norgestomet in conjunction with estradiol valerate to induce estrus in prepuberal beef heifers in a series of experiments. They were able to induce estrus in approximately 93% of the heifers treated with either the standard Syncro-Mate B treatment (5 mg estradiol valerate and 3 mg norgestomet injected im given simultaneously with ear implant containing 6 mg norgestomet left in place for 9 d) or daily im injections of 20 mg progesterone for 4 d plus 2 mg estradiol-17 β 2 d after the last progesterone injection. Pregnancy rates ranged from 43% to 73%. This treatment appeared to be very effective in inducing estrus, and acceptable conception rates were obtained. However, in the 13 yr since this experiment, no one has duplicated these results and the procedure has not been adopted commercially. Perhaps heifers subjected to this treatment need to be near

the time of cycling, and of adequate age and body condition to respond to treatment.

Treatment of 13 to 15-mo old prepuberal heifers with Syncro-Mate B was equally effective when given with or without GnRH in inducing estrus (88% of treated heifers vs 10% controls in 5 d) (Spitzer, 1982), but pregnancy rates over the 5 d breeding period were low (9% for controls versus 30% for treated heifers). Control heifers in these experiments had an estrous response of 48% in 21 days, whereas 91% of the treated heifers exhibited estrus in the same period. Treated heifers did not appear to continue to exhibit estrous cycles. In a similar study, five experiments were conducted in which a total of 393 Holstein heifers were synchronized using Syncro-Mate B (Anderson et al., 1982). Treatment of heifers with estradiol-17 β at the time of implant removal, or gonadotropin releasing hormone 40 h after implant removal did not exert any beneficial effect on fertility than treatment with Syncro-Mate B alone. In addition, conception rates of untreated heifers inseminated over a 25-d period tended to be higher than any of the treated groups. Beal et al.(1984) showed that the Syncro-Mate B treatment or a 9 d-norgestomet implant given in conjunction with a 5 mg im injection of alfaprostol 24 h before implant removal could induce estrus in heifers (77%) with conception rates of 47%. Most noncyclic animals

responded to the treatment, but failed to become pregnant and continued to cycle normally.

Overall, it appears that estrus can be induced in prepuberal heifers. Some heifers ovulated and continued to cycle but others had abnormal cycles or failed to return to estrus after estrogen or progesterone treatments given separately or together.

INHIBIN

Inhibin is a non-steroidal hormone that exerts a specific negative feedback effect on pituitary secretion of FSH in both sexes. There appears to be at least two entities, one is of large molecular weight, apparently between 40 and 70 kDa found in gonadal extracts, and a smaller material between 5 and 20 kDa found in seminal plasma (deJong and Robertson, 1985). In the female, inhibin is produced predominantly in the granulosa cells of ovarian follicles in several species including the cow, sheep, pig, monkey, and man (Tsonis et al., 1983). Concentrations of inhibin measured by an in-vitro pituitary cell culture system using FSH as an endpoint, was unrelated to follicular atresia, but was correlated positively with follicular fluid volume of individual follicles. The same research showed aromatase activity in granulosa cells and estradiol-17 β concentration of follicular fluid (considered to be good

indicators of follicular status) to be highly correlated with inhibin concentrations in follicles larger than 3.5 mm diameter. The ability of bovine granulosa cells to produce inhibin was shown to increase with increasing follicle size in healthy follicles (Henderson et al., 1984). Bolt et al. (1987) demonstrated greater inhibin activity in follicular fluid from large versus small bovine follicles and its inhibin activity was affected by stage of cycle.

Little is known about the regulation of ovarian inhibin production. However, Henderson and Franchimont (1981) showed bovine follicular granulosa cells produced inhibin in vitro, providing the culture medium contained serum. The stimulatory factors are unlikely to be gonadotropins, as bovine LH and/or FSH failed to stimulate inhibin production when added to culture medium devoid of serum. These researchers also showed that inhibin production was stimulated by the addition of androgens to the culture media, was inhibited by the addition of progesterone, and unaffected by the presence of estrogens. No detectable amounts of inhibin were produced by cultured ovarian stromal or luteal tissue (Henderson and Franchimont, 1983). These researchers also showed that follicular tissue produced inhibin in vitro, whereas removal of granulosa cells from the follicle wall resulted in decreased inhibin production by up to 80%.

The major site of inhibin production in the bovine ovary appears to be the granulosa cells of healthy growing follicles and its production appears to be modulated by the gonadal hormones.

INHIBIN LEVELS DURING THE ESTROUS CYCLE AND PERIPUBERTAL PERIOD

Researcher examining the effect of endogenous inhibin on reproductive events has been confined largely to the use of laboratory animals, and the role of inhibin has been implied largely through indirect evidence. In the female rat, there is inhibin-like activity of ovarian venous plasma that varies inversely with FSH concentrations during the estrous cycle (dePaolo et al., 1979). In addition, Welschen et al. (1980) have shown that the injection of steroid-free bovine follicular fluid, which contains inhibin-like activity, suppressed FSH to baseline values in the female rat. Sander et al. (1984) showed that inhibin in the rat appears to be secreted at a higher rate from the granulosa cells collected on the day of proestrus than on any other day of the cycle. However, transport of inhibin from the follicle to the peripheral circulation must be impeded, because that increase in inhibin-like activity was not associated with reduced FSH on proestrus. Inhibin-like activity was not found in the homogenates of ovaries from

immature female rats until 18 d of age. The first significant rise was seen on d 23, and a further rise occurred between d 28 and 33 of age, which is near the time of puberty (Sander et al., 1985). A physiological role for inhibin as a regulator of FSH secretion seems to be present from at least 23 d of age, and may account for the decreasing FSH concentrations seen during the late prepubertal period. The level of inhibin-like activity in late prepubertal female rats was relatively constant from 10 to 5 d prior to their first ovulation, and increased significantly to a peak on the day before their first ovulation (Sander et al., 1986). A significant correlation was found between ovarian inhibin-like activity and the total volume of follicles. FSH concentrations decreased from d -10 to -1 prior to ovulation, thus inhibin probably plays a role in the fine regulation of FSH that is secreted during the peripubertal period.

Treatment with charcoal-extracted follicular fluid is selective in suppressing FSH, because LH is relatively unaffected by treatment. Studies, examining the roles of estrogen and progesterone in the feedback regulation of FSH during the ovine estrous cycle, found that following ovariectomy, replacement therapy of the steroid hormones at physiological levels failed to maintain physiological concentrations of FSH, although some suppression by steroids was evident (Goodman et al., 1981). Concentrations of LH in

the ovariectomized ewes receiving estradiol and progesterone were similar to those of intact ewes, indicating that an ovarian hormone other than progesterone or estradiol is probably involved in the regulation of FSH secretion during the cycle.

Suppression of FSH by steroid-free follicle fluid is apparently independent of the hypothalamus and LH-RH release in the female rat (deGreef et al., 1983). After administrating an inhibin-rich fraction of bovine follicular fluid, LH-RH release into the hypophyseal portal blood was unaffected, whereas plasma levels of FSH were specifically suppressed. Metabolic clearance rates of FSH, LH and prolactin also were unaltered by treatment with follicular fluid. Inhibition of FSH secretion by inhibin was therefore determined to be a direct effect of inhibin on the adenohypophysis, suppressing the release of FSH. In the ewe, treatment with charcoal-extracted follicular fluid reduced FSH concentrations both in the peripheral circulation and in the pituitary (Martin et al., 1986), but did not affect pituitary LH concentrations. In another trial, the same researchers treated ovariectomized ewes with bovine follicular fluid for 21 d. Treatment abolished the increase in FSH seen in control ewes, and maintained concentrations of FSH below those observed in intact ewes. Concentrations of LH rose following ovariectomy and were only partially inhibited by follicular fluid treatment.

Response of these ewes to a GnRH agonist resulted in large surges of LH and FSH in control ewes, but the FSH response in the follicular fluid-treated ewes was completely blocked, and the initial phase of LH release was reduced. This indicated that follicular fluid blocked the secretion of FSH by inhibiting the pituitary response to GnRH, an effect which may result in part from decreased content of FSH in gonadotrophs. Treatment of ovariectomized ewes, which had undergone a hypothalamic-pituitary disconnection, with ovine follicular fluid resulted in a 95% reduction in concentration of FSH and a 32% reduction of LH pulse amplitude compared to controls, after gonadotropin secretion was reestablished with pulses of GnRH (Clarke et al., 1986). Again this indicated that inhibin was effective at the level of the pituitary in inhibiting FSH secretion, and may have some effect on LH secretion.

EFFECTS OF EXOGENOUS INHIBIN ON REPRODUCTIVE EVENTS

The suppression of FSH by the inhibin portion of follicular fluid was observed in domestic farm animals by inhibiting of follicular growth with injections of charcoal-extracted follicular fluid. Treating intact ewes and heifers with bovine follicular fluid following prostaglandin $F_{2\alpha}$ induced luteolysis, resulted in longer intervals to estrus than for ewes and heifers given prostaglandin $F_{2\alpha}$

alone (Miller et al., 1979). Laparotomy of ewes and palpation of heifers after treatment with prostaglandin revealed no size differences in the diameters of the subsequent corpora lutea. After 8 d of treatment with follicular fluid, laparotomy of ewes indicated significant follicular inhibition in both the diameter of the largest follicle and the number of large follicles. It was postulated, therefore, that the delay in return to estrus was due to inhibition of follicular development rather than an interference of luteolysis. Bergfelt and Ginther (1985) also noted a similar follicular inhibition in mares treated with equine follicular fluid.

Charcoal extraction of follicular fluid removes the steroids present, thus removing any attributable effects of steroid hormones contained in the follicle fluid on FSH concentrations. It has been well documented that treatment with charcoal-extracted follicular fluid selectively depressed peripheral FSH concentrations in the rat (Hermans et al., 1980), mare (Bergfelt and Ginther, 1985; Miller et al., 1981), ewe (Miller et al., 1982; McNeilly, 1984; Al-Obaidi et al., 1985; Clarke et al., 1986; Martin et al., 1986; Larson et al., 1987;), and cow (Ireland et al., 1983; Kiracofe et al., 1983; and Johnson and Smith, 1985). In contrast, there was no effect on peripheral LH levels following treatment with charcoal-extracted follicular

fluid. Photoperiod and sex have no influence on the inhibitory effect of follicular fluid on FSH secretion, because the effects of follicular fluid on FSH suppression were similar in gonadectomized male and female sheep, in both the anestrus and the breeding season (Findlay et al., 1985).

Further support for the ability of charcoal-extracted follicular fluid to selectively suppress FSH concentrations is seen in the research of Al-Obaidi et al. (1985). They showed that the suppression of FSH by bovine follicular fluid can be eliminated in adult, ovariectomized ewes by immunizing ewes against bovine follicular fluid compared to ewes immunized against bovine serum albumin. A transient increase in ovulation rate and concentrations of FSH in serum also were noted in heifers after active immunization against partially purified ovine follicular fluid (Price et al., 1987). When merino ewes were immunized with a more highly purified source of inhibin obtained through affinity chromatography, their mean ovulation rate increased from 1.2 to 2.3 (Cummins et al., 1986). This finding, along with the observation that total numbers of follicles > 3.5 mm were also increased, indicated elevated endogenous FSH levels in the presence of inhibin immunization. This increase in ovulation rate was again transient, and no effect of treatment was seen on estrous cycle length, number of granulosa cells per follicle, or seasonal estrous

patterns. When inhibin content was measured in ovaries collected from highly fecund booroola merino ewes, it was found to be one third the value seen in control merino ewes (Cummins et al., 1983). Treating ovariectomized ewes of both strains with charcoal-extracted, ovine follicular fluid resulted in similar suppression of FSH. However, the booroola ewes showed FSH suppression 1 d sooner than controls, indicating that the feedback relationship of inhibin and FSH may be set differently in these two strains and might contribute to the differences seen in their ovulation rates.

Having established the relationship between inhibin and endogenous FSH concentrations, and that treatment with charcoal-extracted follicular fluid can suppress circulating levels of FSH in domestic farm animals, it is of particular interest that upon cessation of treatment with follicular fluid, a rebound effect of FSH concentrations occurs if treatments were sufficient to suppress FSH levels (Miller et al., 1982). This rebound in FSH secretion generally occurs within 24 to 36 h after the last injection of follicular fluid. The degree of this rebound of FSH is proportional to the magnitude of FSH suppression achieved during treatment with follicular fluid (McNeilly, 1984; Bergfelt and Ginther, 1985; Johnson and Smith, 1985). Furthermore, rebound values of FSH seem to be a function of

the degree of FSH suppression and the duration of suppression.

SUMMARY OF REVIEW OF LITERATURE

The onset of puberty in beef heifers is affected by many factors including breed, body condition, plane of nutrition, and environmental factors such as temperature and photoperiod. The process of puberty involves the maturation of the hypothalamic-pituitary axis and its corresponding feedback mechanisms. As the negative feedback effect of estradiol-17 β on LH secretion is gradually relaxed, and as the GnRH pulse generator establishes a more mature mode of secretion, LH is released in its adult-like cyclic profile. The prepuberal rises in progesterone in animals in the absence of ovulation, observed by many, appear to be produced by partial luteinization of immature follicles as a result of these initial surge-like releases of LH. This progesterone source has been shown to be nonessential to the puberal process, but may aid in establishing cyclicity through inhibiting inhibin production, which results in increased FSH concentrations available to the ovary.

Natural and synthetic progestogens have proven to be effective in synchronizing estrus in cyclic cattle and have shown promising results in the induction of puberty and/or estrus in prepuberal heifers of adequate size and condition. The synthetic progestogen, Norgestomet, has been the subject

of many studies. When a norgestomet ear implant is used in conjunction with injections of estradiol valerate, estrous response is often increased but pregnancy rates and the number of animals that continue to cycle are often low.

Inhibin is a naturally occurring non-steroidal hormone that is produced by the follicular granulosa cells of the females of many species. Inhibin production is suppressed by adding of progesterone to a granulosa cell culture system suggesting that its secretion is controlled by gonadal steroids. Inhibin's ability to selectively suppress FSH secretion has been well documented and has been shown to be involved with the varying concentrations of FSH during the estrous cycle. The rising levels of inhibin before the onset of puberty in laboratory animals also correlates with decreasing FSH concentrations at this time. Treating domestic farm animals with charcoal-extracted follicular fluid not only results in suppressed FSH secretion, but a rebound of FSH of a magnitude higher than pretreatment values occurring at the cessation of treatment. The degree of this rebound effect of FSH concentrations is a function of both the degree and duration of suppression of FSH during treatment.

An experimental regimen consisting of treatment with charcoal-extracted follicular fluid (to manipulate endogenous levels of FSH) in conjunction with a norgestomet

implant (to control the timing of LH release) may provide a method of inducing a fertile estrus with high conception rates or establish cyclicity in prepuberal beef heifers.

THE EFFECT OF CHARCOAL-EXTRACTED BOVINE FOLLICULAR
FLUID AND/OR NORGESTOMET ON PREPUBERAL
BEEF HEIFERS

ABSTRACT

Thirty-six crossbred beef heifers which were classified as prepuberal had not exhibited estrus for 6 mo and had low concentrations ($< .6\text{ng/ml}$) of progesterone in each of two pretrial blood serum samples collected 10 d apart. The 36 heifers were allotted into four equal groups in a 2×2 factorial experiment to receive either saline or charcoal-extracted bovine follicular fluid (bFF), 10 ml twice daily im for 4 d, and either a blank or norgestomet-impregnated hydron ear implant for 7 d. Injections started on the same day as implanting (d 0). Four heifers from each group had their ovaries ultrasonographed on d 0, 5, and 12. Heifers were checked for estrus 2 to 4 times daily for 37 d and by d 13, one saline and blank, five saline and norgestomet, one bFF plus blank implant, and seven bFF plus norgestomet-treated heifers had exhibited estrus. A total of four, six, five, and eight heifers had exhibited estrus by d 37 for those same groups, respectively. The number of ovarian follicles $> 7\text{ mm}$ was decreased ($P < 0.05$) with bFF between d 0 and 5, but the number was not affected by norgestomet.

Follicle populations of heifers that had not exhibited estrus were similar on d 12. Concentrations of progesterone measured 10 d after estrus indicated that all heifers had ovulated after exhibiting estrus. However, two bFF plus norgestomet and one of the bFF plus blank implant-treated heifers had a second estrus 2-3 d after their first. The saline and norgestomet-treated heifers exhibited estrus 2 d earlier after implant removal than the bFF plus norgestomet-treated heifers. These data indicate that norgestomet implants induced earlier estrus and ovulation in prepuberal heifers when given with or without charcoal-extracted bFF.

INTRODUCTION

Age at puberty is one of the most influential factors affecting the reproductive efficiency of a cow. Research has shown that a sizable number of beef heifers fail to reach puberty at a desirable age (Laster et al., 1972). Heifers which reach puberty late will either breed late or will not have a chance to be bred. In either case, production efficiency is reduced (Short and Bellows, 1971). Age at puberty can be affected by breed (Joubert, 1968) or plane of nutrition (Oyedipe et al., 1982). These studies indicate that environmental and genetic factors are influential aspects in determining the age at puberty, but provide little insight into the mechanisms involved in the

delayed onset of puberty, an insight that is important if we are to deal with the problems of delayed puberty.

Gonzalez-Padilla et al. (1975a) noted that a rise in serum progesterone concentrations preceded the first estrus in beef heifers, a finding similar to that observed in ewe lambs (Keisler et al., 1983). Progesterone treatment has been shown to induce estrus in prepuberal heifers (Gonzalez-Padilla et al., 1975b, 1975c; Rajamahendran et al., 1982), but conception rates to this induced estrus were often low and variable.

Injections of charcoal-extracted follicular fluid effectively suppresses FSH concentrations in ovariectomized heifers (Ireland et al., 1983; Kiracofe et al., 1983), intact ewes (Larson et al., 1987) and mares (Miller et al., 1982; Bergfelt and Ginther, 1985). As in these last two studies, a rebound of FSH to values greater than pretreatment levels occurs upon the cessation of treatment with follicular fluid. However, no study has reported using this rebound effect of FSH in the induction of puberty. The present study was undertaken to assess the feasibility of norgestomet ear implants in conjunction with a short duration treatment of follicular fluid as a possible method for overcoming the factors involved in the delayed onset of puberty.

MATERIALS AND METHODS

Experimental Animals.

Thirty-six prepuberal Hereford x Angus or Hereford x Brahman heifers, maintained at Kansas State University were utilized. Heifers were 14.1 to 17.2 mo of age and averaged 322 ± 27 (range 265 to 386) kg body weight. Mean weights and ages of heifers were similar at the onset of the experiment (Table 1).

TABLE 1.
COMPARISON OF MEAN AGES AND WEIGHTS OF TREATMENT GROUPS

Treatment Group	Age (Mo.) ^a	Weight (kg) ^a
Saline + Blank	16.2 ^b ±.88	327.29 ^b ±29.55
Saline + Norgestomet	16.0 ^b ±.71	323.56 ^b ±27.49
bFF + Blank	16.1 ^b ±1.0	319.53 ^b ±29.51
bFF + Norgestomet	16.2 ^b ±.78	320.74 ^b ±23.51
Mean	16.1	322.78
SEM	±.84	±27.78

^aMean ± SE

^bMean values in the same column with different superscripts differ at $P < .05$ level.

All heifers were observed twice daily and none exhibited estrus for 6 mo before the experiment. Two pretrial blood samples, collected from all heifers 10 d apart, were assayed for progesterone (Skaggs et al., 1986) and all had concentrations < .6 ng/ml at both bleedings. Both low concentrations of progesterone and lack of estrus were the criteria used to classify heifers as prepuberal. Heifers were maintained together in a drylot and fed prairie hay and protein supplement calculated to meet National Research Council (NRC,1984) requirements for growing, medium frame nonpregnant heifers.

Experimental Design.

A 2 X 2 factorial experiment was conducted to test the efficacy of charcoal-extracted bovine follicular fluid (bFF) and norgestomet on the induction of estrus and ovulation in prepuberal beef heifers. Thirty-six prepuberal heifers were allotted equally by weight and age to receive one of four treatments: 1) a blank implant plus injections of saline (controls), 2) a blank implant plus injections of bFF, 3) a norgestomet impregnated implant plus injections of saline, or 4) a norgestomet implant plus injections of bFF. All injections of saline or bFF were 10 ml, given im and all

ear implants were removed 7 d after implantation. Injections of bFF or saline began at the time of implant insertion (d 0), and were given every 12 h for 4 d. All implants were hydron ear implants^a. All heifers exhibiting estrus during 37 d after implant insertion were inseminated artificially 12 h after detection of estrus, each time they were observed in estrus. All semen used was from a single bull collected during one ejaculation.

Collection and Preparation of Bovine Follicular Fluid.

Follicle fluid was collected by aspirating follicles from ovaries obtained at an abattoir. All follicular fluid was stored at - 20 degrees C until processing. Follicular fluid was thawed and stirred with 50 mg Nordit-A charcoal (Sigma Co., St. Louis, MO) per ml at room temperature for 1 h. The bFF-charcoal solution was then centrifuged at 30,000 x g for 20 min, then filtered through a 0.45-micron pore filter. The bFF was then frozen in 25 ml aliquots until the day of injection.

Ultrasonic Examinations. Four heifers from each treatment group were examined by ultrasonography^b on days 0, 5, and 12 of the experiment to assess ovarian structures. Ultrasonography involved rectal imaging

utilizing a 5 Mhz transducer by an experienced ultrasonic technician. The number and size of follicles on each ovary were recorded. Follicles were classified into categories by diameter: 1) 2-3 mm, 2) 4-6 mm, 3) 7-10 mm, and 4) >10mm. Polaroid pictures were taken of the screen images to further document ovarian structures.

Estrous Detection. Heifers were observed for estrous behavior at 12-h intervals from implant insertion (day 0) until implant removal (d 7). For 5 d following implant removal (d 7 to 12) heifers were checked daily at 0600, 1200, 1800, and 2400 h, then every 12 h from d 12 to 37. A bull with a penis surgically deviated to prevent copulation was used to aid in detection of estrus. Heifers were recorded as exhibiting estrus whenever they stood when mounted by another heifer or the bull. Other signs of estrous behavior such as riding or hyperactivity also were recorded.

Blood Collection. Blood was collected from all heifers at -24, -12, and 0 h (time of implant insertion), before each injection of saline or bFF on d 0 through 4, and at 0600 and 1800 h during d 5 through 12. Blood also was collected from all heifers 5, 10, and 15 d after they were observed in estrus (d 0). All blood samples were

collected by jugular venipuncture. Blood was collected in a 15 ml silicon treated vacuum tube. Blood was refrigerated for 12 h, then centrifuged at $2,000 \times g$ for 15 min. Serum was decanted and stored at -20°C until assayed. Blood collected from -24 h until d 12 after implant insertion was assayed for concentrations of FSH, and blood collected from heifers 5, 10, or 15 d after estrus (d 0) was assayed to determine progesterone concentration.

Progesterone Assay. Plasma progesterone concentration was measured by RIA in 100 μl serum samples after extraction with ethyl acetate (Skaggs et al., 1986). Progesterone concentrations were determined in two assays, with intra- and inter-assay coefficients of variation of 2.0% and 3.0%, respectively. All blood samples were pipetted in duplicate and rerun if duplicates differed by more than 15%.

FSH RIA. Concentrations of FSH in bovine serum were determined by using a double antibody RIA similar to that described by Newton et al. (1987) for porcine FSH with modifications. Purified bovine FSH (USDA-FSH-BP3) was used as the radioligand. Albumin from chicken eggs was added to a pool of filtered bovine serum to give a 5% solution (EA-FBS). Standard curves were prepared in

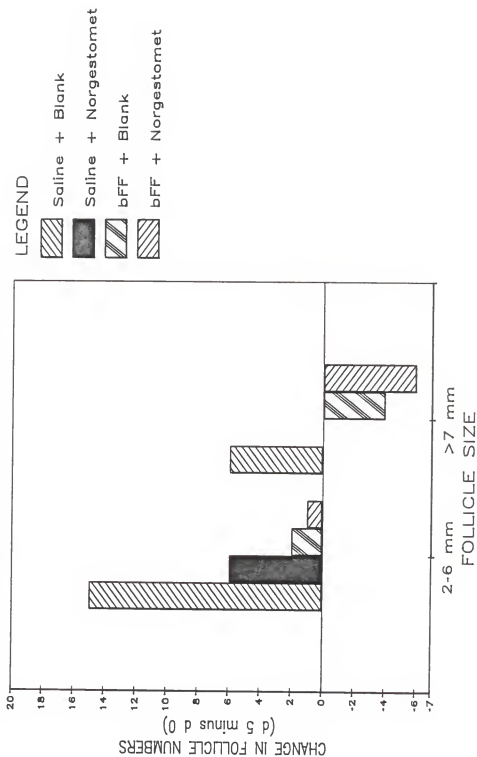
EA-FBS ranging from .1 to 12.8 ng USDA-FSH-BP3/.20 ml EA-FBS. Crossreactivity of the antiserum (Rabbit anti-bovine FSH; Chemicon International, Inc., El Segundo, Ca; Lot #09288) was less than .01% for each USDA-bLH-I-1, USDA-bGH-I-1, NIASDDK-bTSH-I-1, and USDA-bPRL-I-1. Increasing volumes of bovine serum (100, 200, and 300 ml) displaced ^{125}I -labelledbovine FSH from antiserum to produce a binding curve which was parrallel to the standard curve. Sensitivity of the assay was .29 ng/assay tube when incubated for 4 d prior to the addition of ^{125}I -bFSH. All samples were quantified in one assay, with an intra-assay coefficient of variation of 2.0%.

Statistical Analysis. The treatment effects for the proportion of animals in the estrus were compared using chi-square, with no interaction between treatments, the analysis of variance and LSD of pairwise comparisons options of SAS were used (SAS, 1982).

RESULTS

Number and size of follicles for the four heifers from each treatment group that were ultrasonically scanned on d 0 and 5 of the experiment are presented in Table 2. There was no difference in the total number of follicles among treatment groups at the start of the trial (d 0). The number of follicles on d 5 was reduced ($P<0.05$) in the groups treated with bFF than those treated with saline. The total number of follicles was similar in the groups treated with bFF from d 0 to d 5, but the number of follicles in the groups not treated with bFF increased during this time period. Follicles were grouped into two size categories, small (2-6 mm diameter) or large (>7mm diameter). Changes in small and large classes of follicles over the injection phase of the treatment are summarized in figure 1. The largest increase in number of follicles occurred in the saline and blank implant group. This increase represents the addition of 15 small follicles, and six large follicles. Heifers in both of the bFF-treated groups did not experience a significant change in the number of small follicles over the injection phase, but had a decreased number of large follicles. Heifers exhibiting estrus between d 5 and 12 were not scanned on d 12. The average number of follicles on d 12 in

Figure 1. Effect of treatment on follicle numbers. The figure depicts negative and positive changes in large and small follicles by subtracting the number of follicles on day 0 (injections begun) from the number on day 5.



heifers which had not exhibited estrus were not different between treatment groups.

TABLE 2. NUMBER OF OVARIAN FOLLICLES PRESENT ON DAYS OF ULTRASOUND SCANNING

Treatment Group ^a	No. of follicles present by size class (mm)								
	Day 0 ^b			Day 5 ^c			Day 12 ^d		
	2-6	>7	Total	2-6	>7	Total	2-6	>7	Total
S + B	15	5	20	30	11	41	12	4	16
S + N	23	5	28	29	5	34	17	5	22
bFF + B	14	5	19	16	1	17	19	9	28
bFF + N	15	8	23	16	2	18	7	0	7

^a S = saline; B = blank implant; bFF = bovine follicular fluid; N = norgestomet implant; nine heifers in each group.

^b Day 0 = Day the heifers were implanted.

^c Day 5 = 5 d after heifers were implanted and 1 d after end of bFF treatment.

^d Heifers not exhibiting estrus were by d 12 were ultrasonographed (S + B = 2; S + N = 2; bFF + B = 4; bFF + N = 1).

The occurrence of estrus after treatment is shown in Table 3. More ($P < 0.05$) of the norgestomet-treated heifers had exhibited estrus by d 12 than those treated with with blank implants. Day 12 was chosen as a endpoint to assess immediate initiation of cyclicity compared to a delayed effect. Others have shown that in cycling heifers given prostaglandin that bFF delays the onset of estrus about 7 days (Miller et al., 1979). Thus, most heifers induced to cycle by the treatment

should have exhibited estrus by this d 12. The number of heifers exhibiting estrus by 30 d after implant removal was similar, but the norgestomet-treated heifers continued to maintain the advantage shown in the first 5 d after implant removal. Of the heifers exhibiting estrus within 5 d following implant removal in the two norgestomet treated groups, the heifers treated with saline plus norgestomet exhibited estrus 2 d earlier after implant removal than those given bFF plus norgestomet.

TABLE 3. OCCURRENCE OF ESTRUS AND CONCEPTION

Treatment group ^a	No. in estrus/conceiving		Day of trial of onset of estrus ^c
	d 12 ^b	d 37 ^b	
S + B	1/1	4/2	<u>8</u> , 17, 19, 24
S + N	5/3	6/3	8, 10, <u>10</u> , <u>10</u> , 10, 28
bFF + B	1/1	5/3	<u>13</u> , 20, <u>20</u> , <u>20</u> , 24
bFF + N	7/3	8/5	<u>10</u> , 10, <u>12</u> , 12, 12, <u>13</u> , <u>13</u> , <u>29</u>

^a S = saline; B = blank implant; bFF = bovine follicular fluid; N = norgestomet; nine heifers in each group.

^b Day heifers were given implant = d 0; implants were in place for 7 d. Pregnancy determined by rectal palpation 55 to 75 d after insemination.

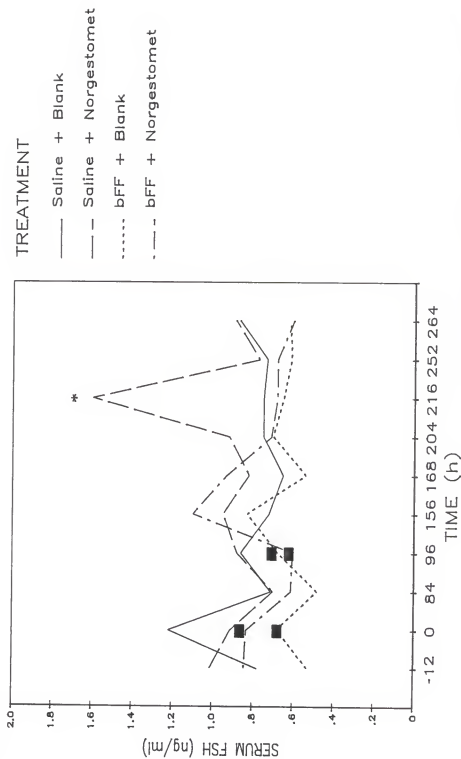
^c Each number represents the day of the experiment when a heifer in that group exhibited estrus. Number underlined indicates the heifer exhibiting estrus on that day conceived.

All heifers which failed to conceive to artificial insemination all had progesterone concentrations above 1 ng/ml 10 d after estrus, indicating that a normal luteal

phase had occurred. Two bFF plus norgestomet and 1 of the bFF plus blank implant treated heifers exhibited a second estrus 2-3 d following their first estrous period, and had elevated progesterone concentrations (above 1 ng/ml) on d 10 following this second estrus period. Conception rates between treatment groups over a 30-d breeding season were similar (Table 3).

Hormone Assay Results. All heifers had elevated concentrations (>1 ng/ml) of progesterone in their serum 10 d after estrus, indicating that ovulation had accompanied estrus. Concentrations of FSH in serum were similarly low in all treatment groups at the onset of the experiment (Figure 2). Treatment with bFF for 4 d tended to depress concentrations of FSH below those at 0 h (Figure 3). Norgestomet did not affect the degree of suppression of FSH expressed in the bFF treated heifers, or the magnitude of the rebound response of FSH concentrations following cessation of treatment with bFF. The magnitude of the rebound of FSH values was to concentrations approximately twice that of FSH values during the injection phase of the treatment (Figure 3). Of the bFF treated heifers, heifers which exhibited estrus after implant removal tended to have a slightly higher degree of both suppression and rebound of FSH concentrations as compared to nonestrous heifers (Figure 4).

Figure 2. Effect of treatment on FSH levels. The figure depicts concentrations of FSH of the four treatment groups from -12 to 264 h. Injections of bFF occur between blocks.



* Peak at 216 h was exaggerated by 3 ng/ml FSH in one halper.

Figure 3. Concentrations of FSH in serum of heifers treated with either saline or bFF. Injections of bFF occurred between blocks.

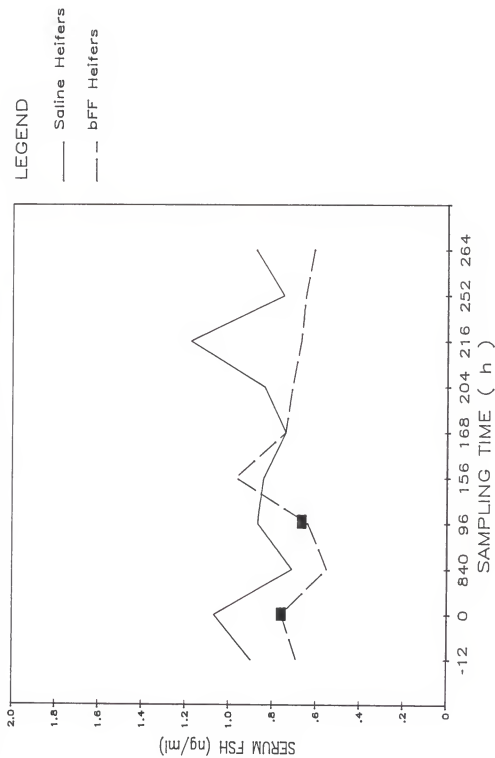
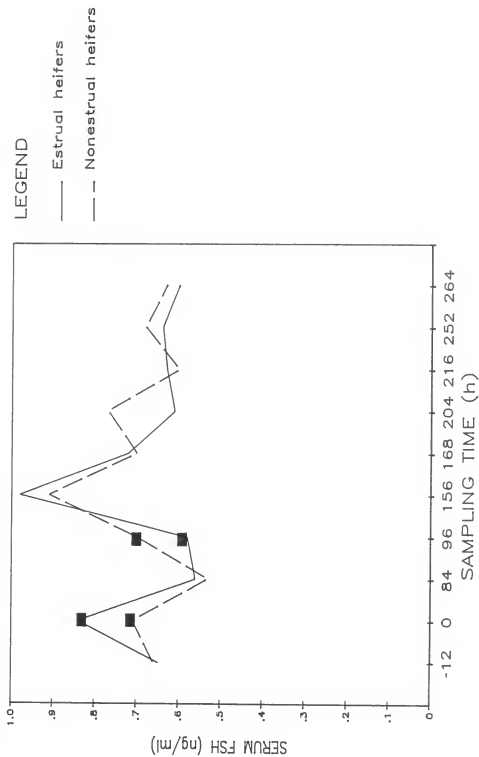


Figure 4. Concentrations of FSH in heifers exhibiting estrus vs heifers not exhibiting estrus after treatment with charcoal extracted bovine follicular fluid. Injections of bFF occurred between blocks.



DISCUSSION

Heifers used in this study were of adequate size and age for their breed type to have attained puberty (Joubert, 1963), and by experimental design, there were no differences in age and weight between treatment groups. Norgestomet apparently inhibited the growth of small follicles into the larger size class, compared to the control heifers. Also, follicular growth was inhibited in both groups receiving bFF. Those groups had little or no change in the number of small follicles but exhibited a significant reduction in the number of large follicles. It appeared that injections of follicular fluid reduced the number of large follicles, presumably by reducing release of FSH from the pituitary. Apparently the control heifers were experiencing an increase in the number of follicles during this period. Norgestomet appeared to prevent the formation of large but not small follicles, and bFF actively reduced the number of large and small follicles (figure 1).

The delayed interval to estrus after treatment with bFF was probably due to the lower number of large follicles in the bFF treated heifers, resulting in an increase in the time necessary for a follicle to reach ovulatory size. A similar delay to estrus has also been

noted in bFF treated ewes and heifers following prostaglandin F2 alpha induced luteolysis (Miller et al., 1979). A prolonged interval to estrus as compared to controls was also note in mares following treatment with bFF (Ginther et al ., 1985). The repeat estrus exhibited by one bFF and two bFF and norgestomet treated heifers, 2 to 3 d after their first, has also been observed by others after the induction of puberty in heifers with Syncro-Mate B (Short et al., 1976). All three heifers were bred on both at both estrous periods and one conceived to artificial insemination. The norgestomet-induced estrus was fertile as indicated by 6 of 12 heifers conceiving to first service within 6 d after implant removal. The norgestomet treated heifers maintained a higher pregnancy rate throughout the 30 d breeding period although no significant differences existed in conception rates between the treatment groups at the end of the breeding period.

Progesterone concentrations >1.0 ng/ml measured 10 d after estrus indicated that estrus was accompanied by ovulation in all hieifers. Though treatment with bFF tended to suppress circulating levels of FSH, neither the degree of suppression or the magnitude of the rebound of FSH values were statistically significant. This might be due to an insufficient dosage or frequency of treatment of bFF, but this treatment did suppress FSH

in bulls (Kiracofe et al., 1988). More likely, the absence of the desired response in FSH values are the result of concentrations that are already quite low, with further suppression of the values being difficult to obtain. It is likely that near the time of puberty in farm animals, as in laboratory species (Sander et al., 1986) endogenous concentrations of inhibin are high approaching the time of first ovulation. These high inhibin levels could account for the low FSH values observed, and might mask the effects of the exogenous follicular fluid treatment.

It appears that treatment with norgestomet was effective in inducing a significantly earlier estrus and ovulation in prepuberal heifers as compared to nontreated controls. Fertility in these heifers was not adversely affected by treatment with norgestomet, and it appears to be an effective means of inducing puberty in heifers of adequate size and age. Treatment with charcoal extracted bFF did not affect induction of puberty, but prolonged the interval to estrus in norgestomet treated heifers apparently by reducing the number of follicles > 7 mm. Future research needs to examine the endogenous levels of inhibin in domestic farm animals around the time of first ovulation, to further clarify this hormone's role in the transition to sexual maturity.

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LITERATURE REVIEW

ESTROUS SYNCHRONIZATION IN BEEF CATTLE.

Estrus synchronization is a practical management tool which allows beef producers to take greater advantage of superior genetics through artificial insemination. Estrus can be successfully synchronized in cattle through the use of progestogens, prostaglandins, progestogen-prostaglandin combinations, and progestogen-estrogen combinations.

The only commercially available progestogen-estrogen combination is Syncro-Mate B (SMB). This treatment consists of a 9-d implant containing 6 mg norgestomet, given simultaneously with an injection containing 5 mg estradiol valerate and 3 mg norgestomet. This product usually results in a high degree of synchronization, but conception rates following its use are often variable and considered to be below the acceptable level. Five trials were conducted using these dosage levels in 744 beef heifers to determine the effectiveness of this treatment in synchronizing estrus (Spitzer et al., 1978). This study also made use of an approximately equal number of controls. The five trials resulted in an average of 95.4% of the treated animals exhibiting estrus during 5 d after implant removal. First-service pregnancy rates were similar to

controls in three of the five studies. In contrast, two studies resulted in more ($P < 0.05$) of the control heifers (21% and 25%) becoming pregnant after the first service. In another study (Anderson et al., 1982), only 88% of the heifers given the Syncro-Mate B treatment were detected in estrus 5 d after implant removal, and conception rates were similar to that of controls. Giving estradiol-17 β at the time of implant removal or GnRH 40 h later did not further improve fertility.

Some of the variability in conception rates following synchronization with Syncro-Mate B is associated with the stage of the cycle in which treatment is initiated. Brink and Kiracofe (1988) reported that cows treated with Syncro-Mate B at early stages of the estrous cycle had higher conception rates than cows treated at later stages of the cycle. Heifers treated with Syncro-Mate B also exhibited similar responses. In a trial conducted by Mikeska and Williams (1988), however, pregnancy rates were similar in cows treated either early or late in their estrous cycles. Spitzer et al. (1978) noted that heifers which failed to show estrus after treatment with Syncro-Mate B were either in the very early or late stages of the cycle. Another factor involved in the sometimes less than satisfactory conception rates observed after treatment with Syncro-Mate B, could be the lack of synchrony in

the normal preovulatory events. Mikeska and Williams (1988) noted that in cows treated with Syncro-Mate B, those which experienced either a delay in the onset of estrus (> 54 h after implant removal) or a delayed LH surge (> 12 h after estrus) had a conception rate of 10%, and this occurred in 19 to 28% of the animals in their study. Kazmer et al. (1981) also noted that following treatment with Syncro-Mate B, intervals from implant removal to estrus and from implant removal to peak LH release were highly variable. Erb et al. (1976) noted that in untreated cows, a consistent difference observed among cows with nonfertile inseminations was delayed estrus and a delayed preovulatory increase in LH after progesterone concentrations had decreased to less than .75 ng/ml.

Syncro-Mate B and similar estrogen-progestogen treatments may have some application in inducing estrus in anestrus cows and prepuberal heifers, even though conception rates also have been variable. Beal et al. (1984) reported that 78% of postpartum lactating cows and 88% of heifers treated with Syncro-Mate B were observed in estrus 5 d after implant removal. More animals cycling before treatment (88%) were detected in estrus than those which were anestrus prior to treatment (77%). Pregnancy rates after 5 d were similar between heifers which were either cycling or anestrus

prior to treatment, but pregnancy rates of anestrous cows were lower than cycling cows (17 vs 55%). Although treatment induced estrus in both cycling and noncyclic animals, marked variability existed in both estrous response and pregnancy rates. Zaied et al. (1976) found similar results in a study in which treatment with Syncro-Mate B induced estrus in 65% of anestrous cows but conception rates tended to be lower than those of cycling cows (31% vs 52%). Treatment with GnRH, in addition to Syncro-Mate B, failed to improve conception rates.

In a study by Spitzer (1982), treatment with Syncro-Mate B induced estrus in a percentage of light weight peripubertal heifers, but pregnancy rates were very low and a large proportion of these heifers, which failed to conceive to the induced estrus, failed to continue cyclicity. Treatment with GnRH following treatment with Syncro-Mate B reduced conception rates in these heifers. Short et al. (1976) reported that although treatment induced estrus in a similar number of puberal and prepuberal heifers, the pregnancy rate to the induced estrus was lower for the prepuberal heifers. The authors speculated that some of the estrous behavior may have been induced by the estrogen (5 mg) because estrus can be induced in spayed cows with as little as 500 μ g estradiol-17 β (Cook et al., 1987).

Induced Estrus in Ovariectomized Cows

Many researchers have been involved in investigating the levels of gonadal hormones required to induce estrous behavior. Much of this work has been accomplished through utilizing the ovariectomized cow or heifer as a model, and in some instances, the freemartin heifer. Use of this model allows for quantifying levels of hormones necessary to elicit estrus through replacement therapy, in the absence of the gonadally produced hormones.

Several estrogenic compounds have been used to induce estrus in cattle. One of these compounds, estradiol benzoate, was utilized in a trial involving dairy heifers (Asdell et al., 1945). Two to three year-old heifers, 35 d after ovariectomy, were used to determine that a minimum of 600 rat units daily, for 3 d, was required to induce estrus. Estrus lasted less than 1 d in spite of continuously increasing daily dosages (up to 10,000 rat units). These researchers postulated that as estrogen levels reach a threshold level the cow exhibits estrus for a normal duration, then an "estrus block" occurs, apparently in the central nervous system, so that the cow is out of estrus before ovulation.

In another study involving the use of estradiol benzoate to induce estrous behavior, 60 ovariectomized beef heifers (30 d after ovariectomy) were involved in two trials studying the effect of varying doses of estradiol benzoate and hCG on induced estrous behavior (Ray, 1965). The dosage levels of estradiol benzoate which were evaluated were 0.2, 0.3, 0.4, 0.5, and 0.6 mg in conjunction with either 0, 2000 or 5000 i.u. of hCG. It was concluded that the minimal dosage of estradiol benzoate sufficient to induce standing estrus was 0.3 mg and the induced estrous period tended to increase with increasing dosage levels of estradiol benzoate. Treatment with hCG had no effect.

Ovariectomized cows and heifers were used to investigate the potential neuroendocrine mechanisms controlling estrous behavior (Cook et al., 1986). Dosage levels ranging from 125 to 4,800 μ g estradiol benzoate were given in conjunction with either 0, 250 or 500 μ g GnRH. The percentage of cows in estrus was lower for those receiving the lower than the higher dosages of estradiol benzoate, with no effect of GnRH injections on estrous response. Interval from injection of estradiol benzoate to the onset of estrus was similar for all treatments. However, the duration of the induced estrus was longer for the cows receiving 4,800 μ g estradiol benzoate than the other treatment groups. This study

suggests that the onset of estrus is dependent upon a threshold level of estradiol, and that once this threshold is reached, estrus is induced and higher doses of estradiol had no effect on interval from injection to estrus, duration of estrus, and the total number of behavioral interactions observed. Three conclusions were drawn by the authors: 1) the threshold level of estradiol benzoate necessary to induce estrus in the majority of ovariectomized cattle was approximately 500 μg ; 2) the endocrine mechanisms responsible for the duration of estrus in the cow did not display a linear dose-response relationship with estradiol; and 3) GnRH did not enhance the induction of estrus when low dosages of estradiol benzoate were administered.

Two trials were conducted in which ovariectomized cows were subjected to weekly injections of 500 μg estradiol benzoate in conjunction with no or increasing levels of cortisol (experiment 1) or dexamethasone (experiment 2) (Cook et al., 1987). Their results indicated that cortisol given with estradiol benzoate did not inhibit estrus in ovariectomized cows and heifers, whereas treatment with estradiol benzoate in conjunction with dexamethasone tended to lower the percentage of cattle exhibiting estrus. However, treatment with dexamethasone did not affect interval from injection to onset of estrus, duration of estrus,

and total numbers of behavioral interactions. In these experiments, 500 μ g estradiol benzoate induced estrus in a high proportion of ovariectomized cattle (>90%), and the duration of estrus was 11 h, similar to cattle having normal estrous cycles.

It was noted that heifers with the most overt estrus prior to ovariectomy required lower doses of estradiol to exhibit behavioral estrus after ovariectomy (Asdell et al., 1945).

Induced Estrus Through Combinations of Estrogen and Progesterone

The effect of progesterone on the estrous response of estrogen-conditioned dairy cows was investigated in a study utilizing different doses of progesterone and frequency of progesterone treatment relative to estrogen treatment (Melampy et al., 1957). Preliminary experiments indicated considerable variation in the degree of estrous response attained by cows receiving the same quantity of estrogen per unit of body weight, and the minimal amount of estradiol benzoate administered in a single dose, which would induce sexual receptivity, was determined for each cow individually. This was considered to be the conditioning or priming dose, and averaged .4 mg estradiol benzoate. Results

indicated a synergistic action of progesterone in all six cows when 1 mg progesterone was administered concurrently or 12 h following a conditioning dose of estrogen. A similar response was noted in four cows receiving the progesterone treatment 24 h following the priming dose of estrogen. However, when the interval was extended to 48 h between hormones, no effect was observed. A similar synergistic action was observed when 1 mg progesterone was administered 12 h before the conditioning dose of estrogen. They concluded that progesterone can induce estrous behavior, including sexual receptivity in the estrogen preconditioned ovariectomized cow, and that maximal synergistic action occurred when progesterone was injected 12 h before, concurrently, or 12 h following a conditioning level of estrogen. Furthermore, it was noted that progesterone in large doses can antagonize estrous behavior in ovariectomized cows injected with a level of estrogen which would otherwise induce sexual receptivity. Estrogen treated cows injected with 30 or more mg of progesterone failed to show signs of estrus.

Ovariectomized ewes, pretreated with 40 mg progesterone, exhibited estrus when induced with 50 to 150 μ g stibestrol (Wodzicka and Tomaszewska, 1963).

The effect of pretreating ovariectomized cows with different levels of progesterone on the occurrence and

intensity of estrus was determined (Davidge et al., 1987). Progesterone was given two times daily for 5 d, then 2 mg estradiol was injected im 72 h later to induce estrus. The doses of progesterone injected resulted in serum concentrations of .3 to 12.3 ng/ml. Concentrations of progesterone returned to <1 ng/ml by the day of peak estradiol concentration (d 9) except when circulating concentrations were above 6 ng/ml. Peak estradiol levels averaged 23.9 pg/ml 12 h after the injection of estradiol. The percentage of cows exhibiting standing behavior at least once during the trial was decreased by progesterone treatment. The time to the first occurrence of standing estrus increased linearly as dose of progesterone increased. The authors concluded that progesterone caused an inhibitory effect on the estradiol-induced estrus behavior in ovariectomized cows, possibly as a result of down regulation of estradiol receptors in the brain, thereby blocking the action of estradiol. In two groups of ovariectomized heifers, the median effective doses of estradiol benzoate required to elicit behavioral estrus were found to be 121 and 132 μ g (Carrick and Shelton, 1969). Repeated doses of estradiol benzoate at these doses did not induce a state of refractoriness to the estradiol. Repeated doses of 10 mg estradiol benzoate, however, did induce refractoriness to subsequent doses

of 400 μ g. When refractory heifers were subjected to 5 d of 10 mg progesterone, they exhibited a normal estrus response to 400 μ g estradiol benzoate administered 3 d later. Increasing the period of progesterone pretreatment beyond 5 d did not increase their sensitivity to estradiol benzoate. Progesterone pretreatment of heifers, which were not made refractory to estradiol benzoate, did not increase sensitivity to estradiol benzoate. In fact, up to 7 d after termination of progesterone pretreatment, estrous response to physiological levels of estradiol benzoate was reduced. In addition, the reduction in response was greater when heifers were pretreated with 40 mg progesterone/d than with 10 mg. Despite large variations between heifers in time to onset of estrus and mean duration of estrus, there was a consistent trend for increasing dosages of estradiol benzoate to be followed by a shorter time to onset of estrus and a longer duration of estrus.

LH Release in Ovariectomized Cows

Following ovariectomy, concentrations of LH in serum rise rapidly in the absence of any negative feedback from ovarian steroids (Short et al., 1973). Serum LH rose from 3.1 ng/ml on the day after

ovariectomy to 6.8 ng/ml on d 28, and progesterone treatment from d 14 to d 25 did not affect this rise. Rahe et al. (1982) showed that LH is released in a pulsatile manner in longterm ovariectomized cows. During the pulses, LH increased from 2.5 to 6 ng/ml. The interval between pulses was consistent both within and between d of blood collection for individual cows, leading to the conclusion that each cow has an inherent consistent rhythmic pattern of LH release in the absence of ovarian hormones. Environment can influence the pattern of release of LH in ovariectomized cows (Day et al., 1986), with an annual cycle being seen consisting of highest mean LH values in the spring, and the lowest being observed in the fall.

In this same study, ovariectomized cows implanted with estradiol to maintain low levels of estradiol-17 β , exhibited higher pulse amplitudes resulting in higher mean LH concentrations than controls. However, implanted cows had a lower pulse frequency than controls. Forrest et al. (1981) found that initial increases in serum LH concentrations due to injections of estradiol or estradiol-17 β were found to occur earlier in both cows and ewes that had been treated with estradiol (8 to 9 h), than with estradiol-17 β (12 to 18 h). However, more LH was released in response to treatment with estradiol-17 β than estradiol. Short et al. (1973)

induced LH release in ovariectomized cows through injections of estradiol-17 β . Progesterone pretreatment did not affect LH release or the expression of estrus. In cows treated with either progesterone or cortisol at the time of estradiol injection, LH release was not affected. All cows had an LH peak from 16 to 24 h after estrogen injection. However, Schoenemann et al. (1985) showed that progesterone could prevent an estradiol-17 β -induced LH surge in ovariectomized cows. Progesterone also blocked increases in pituitary concentrations of LH, which occurred 20 h after estradiol injections in controls.

SUMMARY OF LITERATURE REVIEW

Syncro-Mate B is a commercial estrus synchronization product that synchronizes estrus in cycling and anestrous cows, and has shown some benefit in inducing puberty/estrus in prepuberal heifers. Although treatment usually results in a high degree of synchrony, conception rates following its use are often variable and unacceptably low, especially in the cases involving anestrous cows and prepuberal heifers. Day of the estrous cycle when treatment with Syncro-Mate B is initiated has been shown to be a factor involved in some of this variation, along with asynchrony of the normal preovulatory events possibly resulting from treatment of

some cows. Another possible explanation of this variation is a treatment-induced estrus that is independent of the ovary. Injections of 250 μ g or less of estradiol benzoate have induced estrous behavior in the majority of ovariectomized cows, and doses of 10 mg caused a state of refractoriness to normal levels of estradiol. Progesterone treatment of cows made refractory to physiological doses of estradiol can restore the estrous response to physiological doses of estradiol. Progesterone in small amounts given around the time of estrogen administration has been shown to act synergistically in causing an estrous response in the ovariectomized cow, or if given in larger quantities, progesterone can antagonize or effectively block the estrous response.

If the ovariectomized cow model is an adequate representation of the amounts of exogenous estradiol needed to elicit a behavioral estrus, the 5 mg of estradiol valerate contained in the injectable component of Syncro-Mate B is clearly of sufficient quantity to elicit an estrous response that is independent of ovarian function. Estrus could be induced if sufficient quantity of estradiol-17 β remained in peripheral circulation following implant removal and the subsequent decrease in norgestomet concentrations.

THE INCIDENCE OF ESTRUS AND ESTRADIOL-17 β
CONCENTRATIONS IN OVARECTOMIZED COWS TREATED
WITH Syncro-Mate B

ABSTRACT

Mature Hereford X Simmental cows, ovariectomized either just before treatment or about 6 mo earlier were given the standard Syncro-Mate B treatment. Cows were observed for estrus every 6 h for 3 d after implant removal. Blood samples collected 10 d after estrus were assayed for progesterone. Blood serum collected on the day of estrus was assayed for estradiol-17 β . The treatment was repeated three times using the same nine to 11 cows. Three wk to 3 mo elapsed between replicates. When replicates were combined within experiment 1, 16 of 29 (55.2%) ovariectomized cows exhibited estrus. No cow had serum progesterone concentrations above .2 ng/ml indicating that luteal tissue was not present. In experiment 2, the same cows were allotted to receive either: (1) Syncro-Mate B or (2) Syncro-Mate B plus a second 9 d implant given 12 h before removal of the first. Treatments were initiated such that the norgestomet implant was removed from both groups on the same day. Experiment 2 was replicated

three times and cows were allotted in a manner that ensured each cow received both treatments. When the three replicates in experiment 2 were combined, 3 of 15 cows with norgestomet for 9 d, and 3 of 17 cows with norgestomet for 18 d exhibited estrus. In experiment 3, the cows used in experiments 1 and 2 received either:

- (1) 2 ml saline im and a blank implant (controls, $n=3$),
- (2) 5 mg estradiol valerate im in 2 ml plus a blank implant ($n=3$), or (3) Syncro-Mate B ($n=4$). Implants were left in place for 9 d. Blood samples were taken at -48, -24, -6, 6, 18, and 30 h (injection and implant=0 h). Samples were collected daily from d 2 to d 9, and then four times daily through d 12. Concentrations of estradiol in serum were higher ($P<0.05$) in both treatment groups than in controls until d 6; thereafter they were not different. Concentrations of estradiol-17 β in serum were similar between the estradiol valerate and the Syncro-Mate B cows until d 7 and d 9, then the Syncro-Mate B cows had higher ($P<0.05$) serum concentrations of estradiol. In experiment 4, ten cows from the previous experiments were implanted with a norgestomet ear implant for 9 d and received no other treatment. Three cows exhibited a high degree of estrual activity after implant removal and one cow exhibited standing estrus. It was concluded that Syncro-Mate B induced estrus in ovariectomized cows. Estrus

was induced despite the length of the implant period being extended to 18 d. Furthermore, the norgestomet component of Syncro-Mate B may have affected the clearance rate of the injected estradiol valerate, and may also be capable of causing the expression of estrous behavior in ovariectomized cows without estradiol valerate.

INTRODUCTION

Syncro-Mate B is a commercially available estrous synchronization product which utilizes a progestogen-estrogen combination. Use of this product typically results in a high degree of synchronization, often greater than 90% of the cows exhibit estrus by 5 d after implant removal (Spitzer et al., 1978). However, conception rates following the use of this product are often variable and unacceptably low. In an attempt to explain some of this variability in conception rates, we designed four experiments to determine if treatment with Syncro-Mate B was capable of inducing estrous behavior in ovariectomized cows.

MATERIALS AND METHODS

Eleven mature ovariectomized Hereford X Simmental cows were used repeatedly in four experiments. Cows were kept with an intact, sexually experienced bull in dirt lots and were fed a diet of either silage or prairie hay plus a milo-soybean supplement at a level that maintained a body condition score of 6 or 7 (Clarke et al., 1983).

The four experiments were conducted over an 11 mo period. Three weeks to 3 mo elapsed between experiments.

Experiment 1.

Experiment 1 consisted of three trials. Nine cows that were ovariectomized 6 to 7 mo before the start of the first trial were used in the first two trials. These nine cows plus two that were ovariectomized 3 d before the onset of treatment (on d 7 of their estrous cycle) were used in trial 3. All cows in Experiment 1 were given the standard Syncro-Mate B (SMB) estrous synchronization treatment which consisted of a 2 ml im injection containing 5 mg estradiol valerate and 3 mg norgestomet, and a hydron ear implant containing 6 mg

norgestomet. The ear implant was removed 9 d later. Kamar heat-mount detector patches were placed on all cows, and cows were observed for estrus at 0600, 1200, 1800, and 2400 h each day from implant removal (d 9) through d 12. Animals were considered to be in estrus if they stood to be mounted by a herdmate or by the bull. Other signs of estrus such as hyperactivity, riding but not standing, head butting, etc. also were recorded.

Blood sera collected 10 d after estrus in all cows that exhibited estrus were assayed for progesterone. In addition, in trials 2 and 3 blood samples were collected from all cows on the day of estrus and on the same day in cows not exhibiting estrus. Serum from those blood samples was assayed for estradiol-17 β .

Experiment 2. The same 11 cows used in Experiment 1 were allotted randomly to two treatments: (1) SMB or (2) SMB plus a second 9 d implant given 12 h before removing the first implant. Treatments were initiated such that the norgestomet implant was removed from both treatment groups on the same day. Experiment 2 was replicated three times (cows were allotted in a manner that ensured that each cow received both treatments and the sequence of treatments over the three replicates was equal in

both groups). Estrus was observed and blood samples collected as in Experiment 1.

Experiment 3. Ten ovariectomized cows were allotted to receive three treatments: (1) a 2 ml injection of saline given im and a blank hydron ear implant that was removed 9 d later (controls; n=3), (2) 5 mg estradiol valerate in a 2 ml im injection and a blank hydron ear implant that was removed 9 d later (n=3), or (3) the SMB treatment (n=4). Estrous activity was determined as in previous experiments except it began at the time of injection and was continued through day 12.

Blood samples were collected from cows in all groups at -48, -24, -6, 6, 18, and 30 h (injection and implant=0 h). Blood samples were then collected daily at 1200 h from 2 to 9 d after cows were implanted. After implant removal at 1200 h on d 9, samples were collected every 6 h until 2400 h on d 12. All blood samples were assayed for concentrations of estradiol-17 β .

Experiment 4. The 10 cows from Experiment 3 were all implanted with the norgestomet ear implant for 9 d and received no other treatment. Estrous activity was determined as in previous experiments and blood samples were collected from all cows exhibiting estrus on the

day of estrus. Blood samples were assayed for concentrations of estradiol 17-beta.

BLOOD SAMPLE COLLECTION. All samples were collected in 15 ml silicon treated vacuum tubes through a 1 inch 20 gauge hypodermic needle. Blood was refrigerated immediately after collection for 24 h and allowed to clot. Samples were then centrifuged at 2,000 x g for 15 min and serum was collected. Serum was frozen and stored at -20 degrees C until assayed.

Hormone Assays. Concentrations of progesterone were measured by using 100 μ l serum samples extracted with ethyl acetate by radioimmunoassay according to procedures previously validated in our laboratory (Skaggs et al., 1986). Progesterone concentrations were determined in a single assay with an intra-assay coefficient of variation of 5.5%. All samples were pipetted in duplicate and rerun if counts of duplicates differed by more than 15%. Concentrations of estradiol-17 β were quantified by radioimmunoassay in four assays according to procedures previously validated in our laboratory (Skaggs et al., 1986). Intra- and inter-assay coefficients of variation of the four assays for estradiol averaged 10.2% and 19.3%, respectively.

Statistical Analysis.

Concentrations of estradiol on the day of estrus were analyzed using the GLM procedure of Statistical Analysis Systems (SAS). Trial 3 was analyzed as a completely randomized design in a split plot analysis with cow within treatment being the whole plot error term and time as the subplot main effect. Since there was a significant time by treatment interaction, data were analyzed by the GLM procedure of SAS with mean separations conducted within time period.

RESULTS AND DISCUSSION

Experiment 1. Treatment of ovariectomized cows with Syncro-Mate B resulted in 55% of all cows exhibiting estrus (Table 1).

TABLE 1. OCCURRENCE OF ESTRUS IN OVARIECTOMIZED COWS TREATED WITH SYNCRO-MATE B

Trial No.	No. Cows	No. Cows in Estrus	Percent (%)
1	9	3	33.3
2	9	7	77.8
3	11	6	54.5
Total	29	16	55.2

Only basal concentrations of progesterone were detected in blood serum collected 10 d after estrus. Cows were never observed in estrus except after treatment and serum progesterone concentrations never exceeded .2 ng/ml. Therefore, ovariectomy was assumed complete. It is well documented (Ray, 1965; Cook et al., 1986 and 1987) that treatment with various doses and forms of estrogens can induce estrus in ovariectomized cattle. However, we are unaware of any reports of estrus occurring 10 to 12 d after the injection of estrogen as our Syncro-Mate B treated ovariectomized cows. Cook et al. (1987) reported that as

little as 500 μg of estradiol benzoate induced estrus in more than 90% of ovariectomized cows treated. The dose of estradiol valerate administered in the SMB treatment was 5 mg. Thus, it may be possible that clearance rate of 5 mg estradiol valerate may be sufficiently slow such that adequate estradiol was present in circulation at the implant removal 9 d later to induce estrus. Mean concentrations of estradiol-17 β in cows that exhibited estrus were greater ($P < 0.05$) than in those cows not exhibiting estrus ($3.9 \pm .4$ vs $1.5 \pm .7$ pg/ml, respectively). Cows that exhibited estrus in trials 2 and 3 had estradiol-17 β concentrations that ranged from 1.62 to 6.59 pg/ml, whereas those not in estrus ranged from 1.30 to 1.72 pg/ml (Table 2).

TABLE 2. OCCURRENCE OF ESTRUS AND ESTRADIOL-17 β CONCENTRATIONS IN OVARECTOMIZED COWS GIVEN SYNCRO-MATE B

Cow No.	<u>Trial 1</u>	<u>Trial 2</u>		<u>Trial 3</u>	
	Estrus	Estrus	E ₂ -17 β (pg/ml)	Estrus	E ₂ -17 β (pg/ml)
33	no	yes	5.71	yes	6.34
42	no	yes	4.02	no	1.30
56	yes	yes	3.61	no	1.60
59 ¹	---	---	---	yes	5.74
81 ¹	---	---	---	yes	3.05
84	yes	yes	6.59	yes	2.43
127	no	yes	1.74	yes	2.36
139	yes	yes	4.68	yes	1.62
159	no	yes	2.43	no	1.72
213	no	no	1.39	no	--- ²
912	no	no	1.32	no	--- ²

¹These cows were not included in trials 1 and 2.

²Data not available.

The mean duration of estrus in trials 2 and 3 was 14.8 \pm 8.0 h (mean \pm sem). Estradiol-17 β concentrations on the d of estrus were correlated positively with duration of estrus ($r=.707$). The serum concentration of estradiol-17 β , regardless of its source, appears to be related to the expression and duration of estrus after treatment with Syncro-Mate B. Possible sources of estrogen could be residual estradiol from the injection, a metabolite of norgestomet, or extraovarian production from the animal.

Experiment 2. If residual estradiol from the injection was responsible for estrus in Experiment 1, then extending the norgestomet implant period could eliminate the estrous response if the disappearance rate was not associated with the presence of norgestomet. When data from the three replicates were combined, three of 16 cows exhibited estrus in both the standard Syncro-Mate B and the 18-d norgestomet groups. There was no pattern of response within individual cows or within the sequence of treatments (Table 3).

TABLE 3. INCIDENCE OF ESTRUS IN OVARIECTOMIZED COWS WITH A NORGESTOMET IMPLANT FOR NINE OR EIGHTEEN DAYS AFTER AN INJECTION OF NORGESTOMET AND ESTRADIOL VALERATE.

Cow No.	<u>Replicate 1</u>		<u>Replicate 2</u>		<u>Replicate 3</u>	
	Estrus		Estrus		Estrus	
	9 d ^a	18 d ^a	9 d	18 d	9 d	18 d
33	---	no	no	---	no	---
42	---	no	no	---	no	---
56	yes	---	---	yes	no	---
59	no	---	---	no	---	no
81	no	---	---	no	---	no
84	---	no	yes	---	---	yes ^b
127	---	yes	no	---	---	---
139	no	---	---	no	no	---
159	no	---	---	no	no	---
213	no	---	---	no	---	no
912	---	no	yes	---	---	no

^aRepresents number of days when a norgestomet implant was in place after the injection of norgestomet and estradiol valerate.

^bCow dropped from study due to an illness unrelated to the trial.

Regardless of treatment, cows that exhibited estrus had higher ($P < 0.05$) serum concentrations of estradiol-17 β than those not exhibiting estrus ($2.2 \pm .3$ vs $1.1 \pm .4$ pg/ml, respectively). Treatment had no effect on estradiol-17 β concentrations after implant removal ($1.9 \pm .3$ vs $1.4 \pm .3$ pg/ml for the 9-d and the 18-d implant groups, respectively). Extended exposure to norgestomet had no

effect on the number of cows exhibiting estrus. Estradiol-17 β concentrations for cows not exhibiting estrus were similar among treatments ($1.0 \pm .3$ vs $1.2 \pm .6$ pg/ml for the 9 and 18-d groups, respectively). The 18 d norgestomet implant period did not prevent estrous behavior after implant removal, and no difference existed between estradiol-17 β concentrations of cows exhibiting estrus after either the 9 or 18-d implant period.

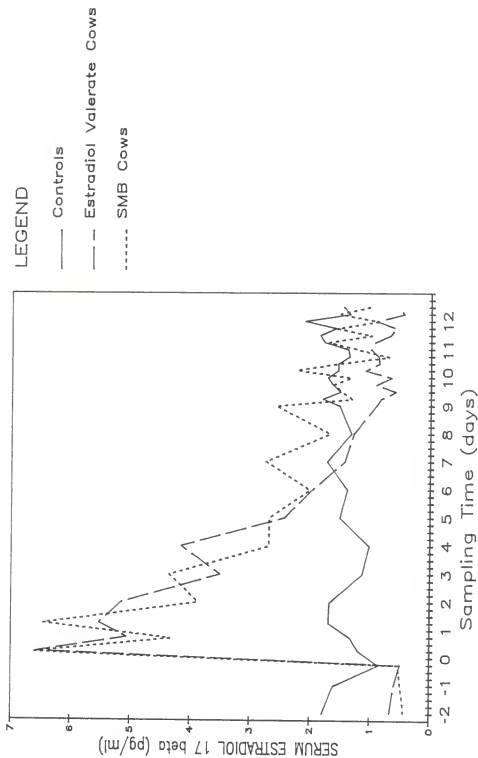
The response to treatment with Syncro-Mate B in the first two experiments was variable. The standard SMB treatment resulted in 0% (0 of 5) to 78.8% (7 of 9) of the treated cows exhibiting estrus. Serum concentrations of estradiol on the day of estrus also were quite variable. Perhaps each cow had an individual threshold concentration of estradiol that was capable of eliciting estrus. Different threshold concentrations of estradiol for inducing estrus in cows were noted by Melampy et al. (1957) and Cook et al. (1986). Extending the norgestomet implant period did not prevent estrus. It would not seem feasible that the injected estrogen could still be present in the serum unless the norgestomet component of the treatment affected the clearance rate of the estradiol valerate.

Experiment 3. To determine if estradiol concentrations in the serum could be altered by the concurrent administration of norgestomet, cows were given either

estradiol valerate alone or in combination with norgestomet (standard Syncro-Mate B-treatment). None of the control cows, 2 of 3 estradiol-valerate treated, and 1 of 4 of the Syncro-Mate B treated cows exhibited estrus. Estradiol valerate treated-cows exhibited standing estrus an average of 96 h after injection (duration 6 h) whereas one Syncro-Mate B-treated cow was in estrus 36 h after implant removal (duration 30 h). Two of the 3 cows receiving the Syncro-Mate B treatment exhibiting estrus showed a high degree of estrous behavior (riding and being ridden, but failing to stand) 3 d before implant removal, but failed to stand. In addition, one of these cows showed a second period of hyperactivity 36 h following implant removal. The one estradiol valerate-treated cow not exhibiting estrus showed a period of hyperactivity at 72 h after injection coinciding with the estrous periods of the two cows that did respond.

Control cows maintained a baseline value of estradiol-17 β that fluctuated between 1 and 2 pg/ml throughout the experiment (figure 1). Both the estradiol valerate and the Syncro-Mate B (estradiol valerate plus norgestomet) treatment groups had higher ($P < 0.05$) concentrations of estradiol-17 β than the controls from d 0 until just before d 6. There was no difference in height of peak, or the time of peak of estradiol-17 β concentrations following either the injection with estradiol valerate or treatment with estradiol valerate plus norgestomet. In the estradiol

Figure 1. Clearance rate of estradiol valerate in ovariectomized cows treated with estradiol valerate or Syncro-Mate B. The figure shows estradiol-17 beta concentrations from -2 d to 12 d (treatment = d 0).



valerate-treated group, estradiol-17 β concentrations declined until they reached baseline values on d 6. On d 6 these values were similar to those of controls. The Syncro-Mate B-treated cows had a more gradual decline (Figure 1) in estradiol-17 β concentrations and on d 7 and 9 were still higher ($P < 0.05$) than controls or cows treated with only estradiol valerate. Blood serum collected on d 9.25 and d 9.5 from Syncro-Mate B-treated cows had higher ($P < 0.05$) concentrations of estradiol-17 β than those taken from the estradiol valerate treated cows, but were not different from controls. The Syncro-Mate B-treated cow that exhibited estrus (Figure 2) had a lower serum estradiol-17 β concentration but maintained a higher concentration of estradiol-17 β during the time the implant was in place as compared to those concentrations of the estradiol valerate treated-cows over the same time frame (Figure 3).

In studies involving induced estrus with estrogenic compounds alone, the interval to estrus was usually 1.5 to 3.5 d after treatment (Carrick and Shelton, 1969, Ray, 1965, and Cook et al., 1986). Our estradiol valerate-treated cows in experiment 3 followed that same trend. Norgestomet was capable of preventing estrus after estradiol valerate because cows did not exhibit estrus until 1 to 2 d after implant removal (11 d after injection). Numerous researchers (Melampy et al., 1957; Carrick and Shelton 1969; and Davidge et al., 1987) have shown that progesterone is

Figure 2. Estradiol valtrate clearance rate in cow no. 84K (SMB). The figure depicts estradiol-17 beta concentrations in the Syncro-Mate B treated cow which exhibited standing estrus. Injection and Implant = d 0.

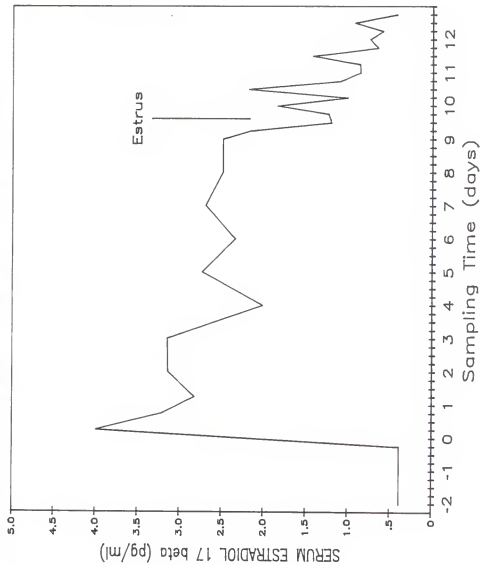
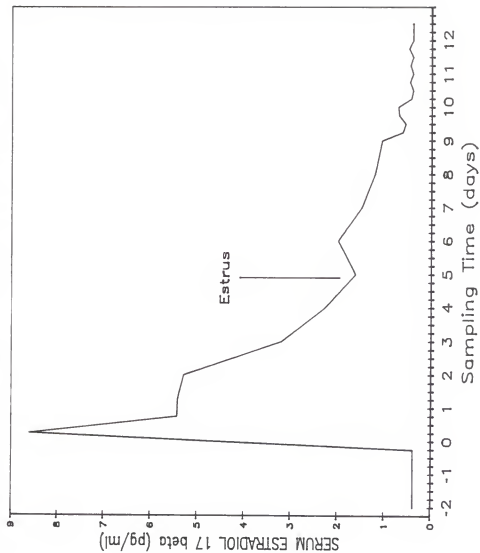


Figure 3. Estradiol valerate clearance rate in cow no. N081 (EV). The figure depicts estradiol-17 beta concentrations in an estradiol valerate treated cow that exhibited standing estrus. Injection (EV) occurred at d 0.



— Cow No. N081

capable of inhibiting estrus in ovariectomized cattle treated with estrogens. However, in those experiments, no estrus occurred after progesterone administration was terminated.

An experiment by King et al. (1974) showed that concentrations of estradiol-17 β returned to baseline by 7 d after injection of estradiol valerate. This is in close agreement with the results of Experiment 3, but fails to explain the elevated estradiol-17 β concentrations in the Syncro-Mate B-treated cows on days 7 and 9. The norgestomet component of the treatment could modify the clearance rate of the estradiol valerate. Estradiol has been shown to be preferentially bound over progesterone by sex hormone binding globulin (SHBG) (Longcope et al., 1987). If this is occurring in the ovariectomized cow after treatment with Syncro-Mate B, the injection of estradiol valerate may be protected from clearance by the presence of the norgestomet. As long as progestin levels are maintained in peripheral circulation (i.e. the implant is in place) estradiol may be bound to SHBG until concentrations of norgestomet decrease, at which time the estradiol may disassociate from SHBG. This possible modification in the clearance rate of estradiol-17 β may explain why higher than expected concentrations of estradiol-17 β occur in ovariectomized cows

as they are receiving norgestomet. If this hypothesis is true, then some cows would be expected to show estrus after norgestomet removal even after extended treatment (18 d) as in experiment 2.

Experiment 4. In the fourth experiment the only treatment was the norgestomet implant. Four of 10 cows exhibited a high degree of estrual activity (riding and being ridden, but failing to stand) after implant removal. However, only one cow exhibited standing estrus. The cow was in standing estrus from 48 to 60 h after implant removal. Blood serum contained .7 pg/ml estradiol-17 β on the day of estrus.

The results of experiment 4 do not seem to support the hypothesis that residual estradiol-17 β from the Syncro-Mate B treatment is the sole triggering mechanism for the expression of estrus. The 9-d norgestomet implant appeared to have sensitized the cows to the point where one was able to exhibit estrus upon implant removal and stimulated hyperactivity in three other cows. It appears that norgestomet either sensitized cows so that they exhibited estrus in response to low levels of extraovarian estrogen, or a metabolite of norgestomet could have estrogenic properties that cause or accentuate estrous-like behavior.

SUMMARY

Syncro-Mate B is a commercial treatment that is effective in synchronizing estrus. However, the percentage of cows conceiving is quite variable after treatment with Syncro-Mate B. The variation in conception rates may be due to a treatment-induced estrus in intact cows which is independent of ovarian function. Data reported from these experiments clearly show that the standard Syncro-Mate B treatment is capable of inducing estrus in ovariectomized cows. However, the percentage of cows responding is quite variable. The treatment-induced estrus in many cases may not be associated with ovulation and improper timing of insemination could result in decreased conception rates.

The mechanism by which Syncro-Mate B induces estrus independent of the ovaries is not clear. Although norgestomet appears to reduce the clearance of estradiol from the blood serum during the 9-d implant period, residual estrogen from the injection does not appear to be the sole triggering mechanism for estrus after implant removal because some cows exhibit estrus after an 18-d norgestomet implant period. This occurs while serum concentrations of estradiol are similar than those of untreated ovariectomized cows. The norgestomet implant may sensitize the cow to extraovarian sources of estradiol and/or residual estradiol

allowing the expression of estrus after implant removal. An alternate possibility is that metabolites of norgestomet possess estrogenic biological activity. Perhaps a combination of any or all of the proposed mechanisms contributed to the expression of estrus. Further research needs to be directed toward explaining the role of norgestomet in the Syncro-Mate B-induced estrus since it appears to be a modulator in the estrous response.

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THE RESPONSE OF PREPUBERAL HEIFERS TO NORGESTOMET
AND/OR FOLLICULAR FLUID AND THE INDUCTION OF
ESTRUS IN OVARIECTOMIZED COWS WITH
SYNCRO-MATE B

by

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ABSTRACT

Thirty-six crossbred beef heifers which were classified as prepuberal had not exhibited estrus for 6 mo and had low concentrations (< 6 ng/ml) of progesterone in each of two pretrial blood samples collected 10 d apart. Heifers were allotted into four equal groups in a 2×2 factorial experiment to receive either saline or charcoal-extracted bovine follicular fluid (bFF) 10 ml im twice daily for 4 d, and either a blank or norgestomet-impregnated hydron ear implant for 7 d. Injections started on the same day as implanting (d 0). Four heifers from each group had their ovaries ultrasonographed on d 0, 5, and 12. Heifers were checked for estrus 2 to 4 times daily for 37 d and by d 13, one saline plus blank, five saline plus norgestomet, one bFF plus blank, and seven bFF and norgestomet-treated heifers had exhibited estrus. A total of four, six, five, and eight heifers had exhibited estrus by d 37 for those same groups, respectively. The number of ovarian follicles > 7 mm was reduced ($P < 0.05$) with bFF between d 0 and 5, but not norgestomet. Concentrations of progesterone measured 10 d after estrus indicated

that all heifers had ovulated after exhibiting estrus. These data indicate that norgestomet implants induced earlier estrus and ovulation in prepuberal heifers when given with or without charcoal extracted bFF. In the second group of experiments, 11 mature Hereford X Simmental cows were used repeatedly in four experiments. In Experiment 1, 55.2 % of the cows exhibited estrus in response to the standard Syncro-Mate B (SMB) treatment. Cows that exhibited estrus had higher ($P<0.05$) serum concentrations of estradiol-17 β on the d of estrus, and no cow had concentrations of progesterone above .2 ng/ml, indicating that luteal tissue was not present. In experiment 2 eleven cows received either: 1) the standard SMB treatment or 2) the standard SMB treatment plus a second 9-d implant. When the 3 replicates of Experiment 2 were combined, the number of cows exhibiting estrus was similar for both groups. Cows exhibiting estrus had higher concentrations of estradiol-17 β than those not exhibiting estrus, and estradiol-17 β concentrations were similar between treatment groups on the d of estrus. In Experiment 3 the same cows received: 1) 2 ml im saline injection and a blank implant, 2) 5 mg estradiol valerate in a 2 ml in injection plus a blank implant, or 3) the standard SMB treatment. Concentrations of estradiol-17 β in serum were similar between the estradiol valerate-treated cows and the SMB-treated cows until d 7 and 9, when the SMB-

treated cows had higher ($P<0.05$) concentrations. In Experiment 4, ten cows from the previous experiments were implanted with the norgestomet ear implant for 9 d and received no other treatment. After implant removal 3 cows exhibited a high degree of estrous behavior and one exhibited standing estrus. It was concluded that Syncro-Mate B induced estrus in ovariectomized cows. Estrus was induced despite the length of the implant period being extended to 18 d. Furthermore, the norgestomet implant may have affected the clearance rate of the injected estradiol valerate, and may also be capable of causing the expression of estrous behavior in ovariectomized cows without estradiol valerate.

KEY WORDS: prepuberal heifers, ovariectomized, norgestomet, induced estrus